BioNanoSci. (2018) 8:319–322
https://doi.org/10.1007/s12668-017-0469-0

Nitric Oxide Production Correlates with Cell Death of Fibroblasts Treated by *Bacillus pumilus* Ribonuclease

Pavel Zelenikhin[1] · **Andreas Koschinski**[2] · **Olga Ilinskaya**[1]

Published online: 8 November 2017

Abstract NO is a crucial messenger in tumor cell signaling. High levels of nitric oxide synthase expression may be cytostatic or cytotoxic for tumor cells, whereas low levels can have the opposite effect and promote tumor growth. Bioimaging is a major technique to visualize the nitric oxide level in living cells and to compare it with physiological outcomes. In this report, we used two fluorescent probes, DAA and DAF-FM diacetate, in order to visualize NO levels in normal and *ras*-transformed fibroblasts treated by the bacterial ribonuclease binase (*Bacillus pumilus* RNase). To assess selective toxicity of binase towards cells expressing the *ras* oncogene, a fluorescent live/dead dye was applied. Here we compared the NO levels in normal and *ras*-transformed fibroblasts to elucidate the role of NO in the apoptotic signaling cascade induced by binase.

Keywords Nitric oxide · Binase · Toxicity · *Ras*

1 Introduction

Nitric oxide and nitric oxide synthases are ubiquitous in malignant tumors and are known to exert pro- and anti-tumor effects [1]. With its rapid synthesis, high permeation and a short half-life, NO is a highly effective molecule with respect to local and transient signaling. NO activity is surprisingly long-termed and more potent in comparison with reactive oxygen species (ROS) [2]. New approaches for live-cell NO visualization were recently developed owing to the important roles of this radical in chemical industry, environmental ecology, and, especially, in biology [3].

Biological signaling through NO is suggested to be mediated by cyclic guanosine monophosphate (cGMP) synthesized by NO-activated guanylyl cyclases. cGMP operates through three main groups of cellular targets: cGMP-dependent protein kinases (PKGs), cGMP-gated cation channels, and cyclic nucleotide phosphodiesterases capable to degrade cGMP [4]. cGMP-binding activates PKG, which phosphorylates serines and threonines in many cellular proteins, and changes activity, functions, subcellular localization, and modulation of regulatory pathways. PKG-dependent regulation of calcium homeostasis and calcium sensitivity of cellular protein modulation is particularly important [4]. Thus, PKG promotes calcium-activated potassium channels opening which leads to cell's hyperpolarization and relaxation and blocks the agonistic activity of phospholipase C, reducing release of stored calcium ions by inositol triphosphate in smooth muscle tissue [5]. Another effect of NO/cGMP/PKG signaling includes PKG-mediated inactivation of the *ras* homolog gene family, member A (RhoA), in many biological processes [4].

Based on these data, we investigated some key molecules important for NO implementation in apoptotic processes, particularly cGMP, Ca^{2+}, and *ras* proteins and their connection with binase-induced apoptosis. Recently, we reported the triple-negative breast cancer BT-20 cell line, carrying a PI3K/AKT-activating mutation of PIK3CA, to be sensitive to apoptotic action of binase [6]. Binase also was shown to induce apoptosis in leukemic Kasumi cells [7], in lung adenocarcinoma cells A549 [8], and the ovarian cancer cell lines SKOV3 and OVCAR5 [9]. The selective cytotoxicity of binase towards cancer cells was partially determined by *ras* oncogene expression [10]. Moreover, binase directly interacts

✉ Pavel Zelenikhin
pasha_mic@mail.ru

[1] Kazan (Volga region) Federal University, Kazan, Russia

[2] Department of physiology, Anatomy and Genetics, University of Oxford, Oxford, UK